Data Summary and Review on the Acute Toxicity of AE C656948 (Fluopyram) to *Pimephales promelas*

PMRA Submission Number N/A EPA MRID Number 473723-31

Data Requirement:	PMRA Data Code	{……..}
	EPA DP Barcode	353315
	OECD Data Point	{……..}
	EPA MRID	473723-31
	EPA Guideline	850.1075

Test material: **AE C656948** **Purity:** 94.7%
Common name: Fluopyram
Chemical name: IUPAC: N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name: N-[2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]ethyl]-2-(trifluoromethyl)benzamide
CAS No.: 658066-35-4
Synonyms: None Reported

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	080302	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Matlock, D and C.V. Lam. 2008. Acute Toxicity of AE C656948 Technical to the Fathead Minnow (*Pimephales promelas*) Under Static Conditions. Unpublished study performed by Bayer CropScience, Ecotoxicology, Stilwell, Kansas. Laboratory report number EBGMP237. Study sponsored by Bayer CropScience, Research Triangle Park, North Carolina. Study completed March 13, 2008.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity test, fathead minnow (*Pimephales promelas*) were exposed to AE C656948 (Fluopyram) at nominal concentrations of 0 (negative and solvent controls), 0.31, 0.63, 1.25, 2.50 and 5.00 mg ai/L; mean measured concentrations were <0.03 (<LOQ; controls), 0.30, 0.57, 1.23, 2.60 and 4.95 mg ai/L under static conditions. The 96-h LC_{50} was >4.95 mg ai/L. The EC_{50} and NOAEC values, based on a lack of mortality or sub-lethal effects, were >4.95 and 4.95 mg ai/L, respectively. Based on the results of this study, AE C656948 would be classified as practically nontoxic to fathead minnow (*Pimephales promelas*) up to the concentration tested in this study, on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified {scientifically sound or unsound} and {does or does not} satisfy the guideline requirement for an acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age (mean weight or length): 0.62±0.11 g (mean wet weight); 41.5±2.6 mm (mean body length)
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >4.95 mg ai/L
NOAEC: 4.95 mg ai/L
EC_{50}: >4.95 mg ai/L
Endpoint(s) Affected: None

95% C.I.: N/A
Probit Slope: N/A

I. REPORTED MATERIALS AND METHODS

GUIDELINE FOLLOWED:
This study and the associated biological methodologies followed guidelines outlined in:

- EPA § 72-1, Acute Toxicity Test for Freshwater Fish (Oct. 1982)/SEP-EPA-540/9-85-006 (June 1985)
- EPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975), EPA-660/3-75-009
- EPA Acquisition and Culture of Research Fish: Sheepshead minnow, Fathead minnows, Channel Catfish and Bluegills (1975), EPA-660/3-75-011
- OPPTS Test Guideline 850.1075, Public Draft: Fish, Acute Toxicity Test, Freshwater and Marine (April 1996)
- OECD Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test (rev. 1992)
- ASTM Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians, ASTM Standard E729

COMPLIANCE:

Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with:

- U.S. EPA-FIFRA Good Laboratory Practice Standards (40 CFR Part 160), with the following exception: Routine spring and reverse osmosis water contaminant screening for pesticides, PCBs, and toxic metals.

A. REPORTED MATERIALS:

1. Test material AE C656948 (Fluopyram)

Description: Light beige powder

Lot No./Batch No. : 08528/0002

Purity: 94.7%

Stability of compound under test conditions: After 96 hours, measured concentrations were 83-109% of nominal, indicating stability under test conditions.

Storage conditions of test chemicals: Room temperature

Physicochemical properties of AE C656948.

Parameter	Values	Comments
Water solubility at 20EC	~5.0 mg ai/L	See Reviewer's Comments
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	

Parameter	Values	Comments
Kow	Not reported	

2. Test organism:

Species: Fathead minnow (*Pimephales promelas*)
Age at test initiation: Juvenile
Weight at study initiation: 0.62±0.11 g (mean ± SD)
Length at study initiation: 41.5±2.6 mm (mean ± SD)
Source: Aquatic BioSystems Inc., Fort Collins, CO

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: No range-finding study was conducted. Nominal concentrations were selected based on the functional solubility of the test material in soft processed water.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details
Acclimation	
Period:	>14 days
Conditions: (same as test or not)	Same
Feeding:	Tetramin and brine shrimp, provided at least once daily
Health: (any mortality observed)	• 0% mortality during 48 hours prior to test • All unsuitable fish (e.g. injured, deformed, etc.) eliminated prior to test group assignment. • No prophylactic or therapeutic pre-treatment of fish
Duration of the test	96 hours
Test condition	

Parameter	Details
Static/flow-through	Static
Type of dilution system - for flow-through method.	N/A
Renewal rate for static renewal	N/A
Aeration, if any	None
Test vessel	
Material: (glass/stainless steel)	Glass
Size:	38 L; 49.5 x 25.4 x 30.5 cm
Fill volume:	30 L; 49.5 x 25.4 x 23.8 cm

Source of dilution water Quality:	Culture and test water was a blended soft water. Blended water is a mixture of RO water blended with spring water to produce soft (40-60 mg/L) water. Spring water was collected from a spring box and passed through a multimedia filter, a 5 micron bag filter, granular activated carbon filters, a 1 micron cartridge filter and an ultraviolet sterilizer. RO water was municipal water that had been dechlorinated with sodium metabisulfite, passed through a multimedia filter, granular activated carbon units, demineralized by conventional softeners, passed through a 5 micron cartridge filter and treated by double pass reverse osmosis.

Water parameters: Hardness	 50-54 mg $CaCO_3$/L
pH	7.6-8.1
Dissolved oxygen	7.3-8.4 mg/L (84-96% saturation)
Total Organic carbon	2.9 mg/L in spring water, <0.50 mg/L in RO water
Particulate Matter	<1 mg/L
Metals	See Reviewer's Comments
Pesticides	None detected
Chlorine	<0.003 mg/L as residual
Temperature	22.1-22.8°C
{Salinity for marine or estuarine species}	N/A
Intervals of water quality measurement	Temperature was measured hourly. pH and DO were measured daily.
Number of replicates/groups: control: solvent control: treated ones:	 1 1 1
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 10 10
Biomass loading rate	0.21 g/L
Test concentrations: Nominal: Mean-measured:	 0 (negative and solvent controls), 0.31, 0.63, 1.25, 2.50 and 5.0 mg ai/L <0.03 (<LOQ; controls), 0.30, 0.57, 1.23, 2.60 and 4.95 mg ai/L
Solvent (type, percentage, if used)	DMF; 0.1 mL/L

Lighting	16 hours light:8 hours dark with a 30 minute transition period
Feeding	Fish were not fed 48 hours before and during the study
Recovery of chemical Frequency of determination Level of quantification Level of detection	 Days 0 & 4 0.03 mg ai/L Not reported
Positive control {if used, indicate the chemical and concentrations}	N/A
Other parameters, if any	N/A

2. Observations:

Table 2: Observations

Parameter	Details
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal behavioral observations
Observation intervals	4, 24, 48, 72 and 96 hours
Were raw data included?	Yes
Other observations, if any	N/A

II. REPORTED RESULTS:

A. REPORTED MORTALITY:

No mortalities were observed in the controls or treatment groups throughout the definitive exposure period yielding NOAEC and LC_{50} values of 4.95 and >4.95 mg ai/L, respectively.

Table 3: Effect of AE C656948 (Fluopyram) on Mortality of Fathead minnow (*Pimephales promelas*).

Mean-Measured and (Nominal) Concentrations mg ai/L	No. of Fish at Start of Study	Observation Period					
		24 Hours		72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control (<0.03 mg ai/L)	10	0	0	0	0	0	0
Solvent Control (<0.03 mg ai/L)	10	0	0	0	0	0	0
0.30 (0.31)	10	0	0	0	0	0	0
0.57 (0.63)	10	0	0	0	0	0	0
1.23 (1.25)	10	0	0	0	0	0	0
2.60 (2.50)	10	0	0	0	0	0	0
4.95 (5.0)	10	0	0	0	0	0	0
NOAEC	4.95 mg ai/L						
LC_{50}	>4.95 mg ai/L						
Positive control, if used mortality: LC_{50}:	N/A						

N/A- Not Applicable

B. REPORTED SUBLETHAL TOXICITY ENDPOINTS:

There were no sublethal effects in the controls or treatment levels.

Table 4: Sub-lethal Effect of AE C656948 on Fathead minnow (*Pimephales promelas*).

Mean-Measured and (Nominal) Concentrations mg ai/L	Observation Period		
	24 Hours	72 Hours	96 Hours
	% Affected	% Affected	% Affected
Negative Control (<0.03 mg ai/L)	0%- A.N.	0%- A.N.	0%- A.N.
Solvent Control (<0.03 mg ai/L)	0%- A.N.	0%- A.N.	0%- A.N.
0.30 (0.31)	0%- A.N.	0%- A.N.	0%- A.N.
0.57 (0.63)	0%- A.N.	0%- A.N.	0%- A.N.
1.23 (1.25)	0%- A.N.	0%- A.N.	0%- A.N.
2.60 (2.50)	0%- A.N.	0%- A.N.	0%- A.N.
4.95 (5.0)	0%- A.N.	0%- A.N.	0%- A.N.
NOAEC	4.95 mg ai/L		
LOAEC	>4.95 mg ai/L		
EC_{50}	>4.95 mg ai/L		
Positive control, if used % sublethal effect: EC_{50}:	N/A		

A.N.- All surviving fish appear normal and healthy
N/A- Not Applicable

C. REPORTED STATISTICS:

The study authors did not perform statistical analysis and based toxicity values on the mean-measured test concentrations.

III. REVIEWER'S EVALUATION

A. DEVIATIONS FROM GUIDELINES: No deviations from OPPTS guideline 850.1075 were noted.

B. OTHER STUDY DEFICIENCIES: None.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Due to the lack of mortality and sub-lethal effects, the reviewer visually determined the LC_{50}, EC_{50} and NOAEC values based on the 96-hour mean-measured concentrations.

LC_{50}: >4.95 mg ai/L 95% C.I.: N/A
NOAEC: 4.95 mg ai/L
Probit Slope: N/A
EC_{50}: >4.95 mg ai/L 95% C.I.: N/A

D. ADDITIONAL REVIEWER COMMENTS:

The reviewer's results were identical to those of the study authors.

No test material was observed in test vessels at any time during the definitive toxicity test; the test medium remained clear throughout the test.

The functional solubility of the test material in the dilution water was determined from multiple solubility trials using identical soft processed water with similar stock solutions and solvent loads. Precipitates were observed in all test solutions at concentrations of 6 to 10 ppm; therefore, the functional solubility was determined to be approximately 5.0 mg ai/L under definitive test conditions.

The analytical method validation yielded an average recovery of 98% with a relative standard deviation (RSD) of 6%. A 0.50 ppm laboratory spike was prepared and analyzed with the test solutions of each sampling interval. Recoveries were 101 and 93% on Days 0 and 4, respectively.

The periodic screening analysis of the spring water indicated the presence of the following metals and inorganics: aluminum (32.0 µg/L), calcium (140,000 µg/L), chromium (8.4 µg/L), magnesium (9,400 µg/L), molybdenum (4.1 µg/L), nickel (1.9 µg/L), potassium (1,300 µg/L), selenium (1.8 µg/L), sodium (52,000 µg/L), zinc (4.6 µg/L), chloride (88 mg/L), fluoride (0.61 mg/L), nitrate as N (0.024 mg/L), nitrate as N Ion Chromatography (4.2 mg/L), total phosphorous (0.027 mg/L), and sulfate (120 mg/L). The periodic screening analysis of the RO water indicated the presence of the following metals and inorganics: aluminum (3.6 µg/L), calcium (19,000 µg/L), chromium (1.7 µg/L), copper (1.7 µg/L), sodium (12,000 µg/L), zinc (1.4 µg/L), chloride (15.0 mg/L), and nitrate as N Ion Chromatography (0.63 mg/L).

The in-life portion of the definitive toxicity test was from January 7 to January 11, 2008.

E. CONCLUSIONS:

This study is/is not scientifically sound and does/does not fulfill the requirements for an acute freshwater fish toxicity study. The 96-hour LC_{50} was >4.95 mg ai/L. Due to a lack of mortality or sub-lethal effects, the NOAEC was 4.95 mg ai/L. Based on the results of this study, AE C656948 would be classified as practically nontoxic up to the limit of solubility to Fathead minnow (*Pimephales promelas*) on an acute toxicity basis.

LC_{50}: >4.95 mg ai/L	95% C.I.: N/A
NOAEC: 4.95 mg ai/L	Probit Slope: N/A
EC_{50}: >4.95 mg ai/L	95% C.I.: N/A
Endpoint(s) Affected: None	

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